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IMMUNISATION AGAINST *B. WELCHII*: TITRATION OF TOXIN AND ANTITOXIN.

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T. DALLING and J. H. MASON.

Wellcome Physiological Research Laboratories.

During the investigation of a diarrhœal disease affecting young lambs, the prevalence of *B. Welchii* in the tissues suggested that this bacillus might be a cause, or one of the causes, of the condition. We therefore decided to use its toxin in an attempt to protect the lambs. Although the ætiology of the disease has not been fully investigated it is of interest that the prophylactic we used definitely produced protection against the disease. 821 pregnant ewes were injected and the mortality amongst their lambs was 3·4 per cent.; 2367 pregnant ewes on the same farms were observed as controls and amongst these the mortality was 23·1 per cent.

For immunisation it was deemed advisable to use a toxin-antitoxin mixture; this was titrated by intramuscular injection into mice, the method referred to in the Medical Research Council Report of the Committee on Anaerobes. We now use the intravenous method which gives consistent results. Two successive recent batches of toxin killed all mice injected with doses from 1 c.c. to 0·01 c.c. in a few minutes, and those receiving 0·005 c.c. in 60 minutes. All mice receiving lower doses lived. The next batch of toxin killed all mice receiving doses down to 0·0006 c.c., one out of two mice at 0·0004 c.c., while all receiving doses lower than this amount lived.

The P_H of these toxins was between 5·5 and 6·0; this was altered with NaOH to 7·8 and the tests on the acid and neutral toxin were carried out in parallel. With one batch under observation, all mice receiving doses of a toxin with a P_H of 5·5 down to 0·003 c.c. died, while all those receiving 0·002 c.c. lived. This result was the same when the P_H of the toxin was 7·8.

It was easy to titrate serum against a good liquid toxin; 0·1 c.c. would protect against at least 5000 M.L.D. the amount we have used as a routine in testing. The neutralisation was specific, for normal horse serum in large doses has in no instance shown any protection. Results similar to those described were obtained by the intravenous injection into the external popliteal vein of guinea-pigs.

Toxin was treated with 0·3 per cent. formalin and incubated at 37° C. It was found that in two to three days the M.L.D. was reduced from approximately 0·02 c.c. for an 18 grms. mouse to 0·5 c.c. Incubation for a week longer did not alter the toxicity further.

We compared the immunising value of (*a*) formalinised toxoid, the M.L.D. of which for an 18 grm. mouse was about 1 c.c.; (*b*) stored toxin, the toxicity of which for mice had altered from 0·006 to 0·1 c.c.; (*c*) toxin-antitoxin mixture in which approximately 12 c.c. of the serum in use was added to one litre of toxin. One dose of (*a*) enabled about 50 per cent. of guinea-pigs, when tested 14 days later, to withstand one certain lethal dose of toxin; another group when tested 21 days after two inoculations, given in a fortnight, survived a lethal dose of toxin in four instances out of five. The results with (*b*) were somewhat similar, four out of five surviving when tested three weeks after the second inoculation of the prophylactic. (*c*) Amongst those given two doses of the toxin-antitoxin mixture, five out of seven survived three weeks after the second dose of the prophylactic. By all three methods a reasonably high degree of immunity can be produced. It is not certain which is the best method, and the experiments are being continued.

Intradermic method.—During the war this method was used by Glenny for titrating toxin and antitoxin. *Welchii* toxin injected intradermically produced intense hyperæmia followed a few hours later by blanching and necrosis. The reaction is specific; the toxin is neutralised by its homologous antitoxin, 0·001 c.c. of an average serum completely neutralising 20 to 30 minimal skin reacting doses.

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